

<p style="text-align: center;">Margosa Extract Evaluation of Classification and Labelling Proposal with regard to Skin Sensitisation</p>

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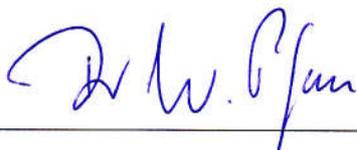
AUTHENTICATION

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Report Title: Margosa Extract: Evaluation of Classification and Labelling Proposal with regard to Skin Sensitisation

I, the undersigned, hereby declare that the present report has been prepared by GAB Consulting GmbH, Hinter den Höfen 24, 21769 Lamstedt, Germany.

Signature



Date

20. November 2014

Prof. Dr. Wolfgang Pfau

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1 Executive Summary

Margosa extract is a refined medium polarity extract from the kernels of the Neem tree. It is approved in the EU as active substance for biocidal products of the product type 18.

RMS Germany prepared a CLH-Dossier to define an appropriate harmonized classification for Margosa Extract and, regarding skin sensitisation, proposed classification in Category 1 (H317).

Based on the submitted data Margosa Extract did meet the criteria laid down in the CLP regulation to be classified with Skin sensitisation Category 1B (H317 - May cause an allergic skin reaction).

2 Introduction

Margosa Extract is a refined medium polarity extract from the kernels of the Neem tree. Margosa Extract was included into Annex I of Directive 98/8 EC by Commission Implementing Directive 2012/15/EU (8 May 2012) for pesticide product type 18 in the EU.

Regarding skin sensitisation, classification in Category 1 (H317) was proposed in the CLH report. The available data on skin sensitisation is discussed in the following with the conclusion that classification with Skin sensitisation, Category 1B is appropriate.

3 CLH-Proposal – Skin sensitisation

In the CLH report (Proposal for Harmonised Classification and Labelling) the RMS Germany concluded regarding classification and labelling that

Results with NeemAzal and NPI 720 lead to a classification in category 1B, whereas results with Fortune Aza lead to category 1A. Considering the contradictory categories, it is proposed to place Azadirachtin into category 1 (without sub categories), see Part B, Point 4.6.1.4 (page 24).

4 Available Data

The rapporteur MS Germany prepared only one toxicological evaluation for both Azadirachtin (CAS No: 11141-17-6) and Margosa Extract (CAS-No: 84696-25-3). This is appropriate since the extract “NeemAzal” is identical for both Azadirachtin and Margosa Extract.

However, while evaluation of the plant protection insecticide Azadirachtin is based on three Azadirachtin Extracts from three different sources (including NeemAzal), the biocide Margosa Extract is identical to NeemAzal only.

Classification is, thus, to be based on experimental data for NeemAzal.¹ An experimental study (Magnusson Kligman test) conducted with NeemAzal according to GLP and the OECD technical guideline 406 has been evaluated in the CLH report and is summarised in Table 1.

¹ The weight of evidence, considering all data available including experimental data showing that the products containing Azadirachtin are lacking any skin sensitising activity, indicates that also Azadirachtin the pesticide is appropriately classified as Skin sensitiser Category 1B.

Table 1: Summary of skin sensitisation (taken from CLH report, Part B, Point 4.6.1.1, Table 19)

Animal species strain	Number of animals	Doses	Result	Reference Method
Guinea pig, Dunkin Hartley albino	20 M treated 10 control	Intradermal: 5 % (w/v) in acetone/alembicol Dermal: 80 % in acetone	Sensitising (M&K) [all animals sensitised] NeemAzal	Allan & Coleman, 1997 TOX9700507 OECD TG 406

Table 2: CLP criteria for skin sensitisation

Guinea pig maximisation test (Magnusson Kligman Test)	Category 1A (H317): ≥ 30 % responding at ≤ 0.1 % intradermal induction dose or ≥ 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose
	Category 1B (H317): ≥ 30 % to < 60 % responding at > 0.1 % to ≤ 1 % intradermal induction dose or ≥ 30 % responding at > 1 % intradermal induction dose

Comparing the test results with the CLP criteria, and in agreement with the evaluation by the rapporteur it is concluded that *Results with NeemAzal [...] lead to a classification in category 1B.*

The classification of Margosa Extract as a weak skin sensitising agent is corroborated by experimental data with the biocidal product containing 3% Margosa Extract (NeemAzal-T/S). This product was demonstrated to lack skin sensitising activity in a Magnusson Kligman test using induction-concentrations of 5% and 30% for intradermal and topical induction, respectively (data summarised in Table 3 below and a more detailed study summary is included in the Appendix).

Table 3 Skin Sensitisation study with the biocidal product

Number of animals (Guinea pigs)	Test substance Concentration of Azadirachtin	Doses product (Induction)	Doses active substance (Induction)	Result	Reference Report No Method
M&K 20 M treated 10 control	NeemAzal T/S 3% Azadirachtin Extract NeemAzal	Intradermal: 5 % in sesame oil Dermal: 100% undiluted	Intradermal: 0.15% Dermal: 4.5%	Not sensitising [no animal sensitised]	Kramer, 1998 981042830 OECD 406

6 Overall conclusion

In summary based on the submitted data and considering also the experimental evidence obtained with the plant protection product, Margosa Extract did meet the criteria laid down in CLP regulation to be classified with Skin sensitisation Category 1B (H317 - May cause an allergic skin reaction).

7 References

Author(s)	Year	Title Testing Facility Owner / Source (where different from owner) Report No GLP or GEP status (where relevant) Published or not	Owner
Allan, S., Coleman, D.	1997	Neemazal technical skin sensitisation in the guinea-pig Huntingdon Life Sciences Ltd., Huntingdon, UK Trifolio-M GmbH Report-no. EIP 10/950818/SS GLP: yes Published: no	TRF
Kramer, H.-J.	1998	Skin Sensitisation Study according to Magnusson & Kligman BioChem GmbH, Karlsruhe, Germany Trifolio-M GmbH Report-no. 981042830 GLP: yes Published: no	TRF

8 Appendix

The following study summary of skin sensitisation study performed with NeemAzal-T/S was taken from the CAR on Margosa Extract of January 2012 showing that this formulations had no sensitising effects.

Section B6.3	Skin sensitisation
Annex Point IIB-VI6.3	Magnusson Kligman Test

1 REFERENCE	
1.1 Reference	Kramer, H.-J. (1997) Skin Sensitation Study according to Magnusson & Kligman NeemAzal T/S BioChem, Karlsruhe, Germany Unpublished Report No. 981042830 Dates of experimental work: 11.09. – 10.10.1998
1.2 Data protection	Yes
1.2.1 Data owner	Trifolio-M GmbH
1.2.2 Companies with letter of access	Not applicable
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I.
2 GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes OECD 406
2.2 GLP	Yes
2.3 Deviations	No
3 MATERIALS AND METHODS	
3.1 Test material	NeemAzal-T/S
3.1.1 Lot/Batch number	Batch No 100898
3.1.2 Specification	
3.1.2.1 Description	Dark brown liquid
3.1.2.2 Purity	1 % Azadirachtin actual concentration 1.15 – 1.21 % Azadirachtin A
3.1.2.3 Stability	Stable at room temperature in the dark
3.1.3 Preparation of test substance for application	<u>for induction:</u> 5 % (intradermal) and 25% topical in sesame oil <u>for challenge:</u> 25 % in sesame oil
3.1.4. Pretest performed on irritant effects	Yes

Section B6.3**Skin sensitisation****Annex Point IIB-VI6.3**

Magnusson Kligman Test

3.2 Test Animals

3.2.1	Species	Guinea pigs	
3.2.2	Strain	Dunkin Hartley (Hra:DH)BR	
3.2.3	Source	Harlan Winkelmann, Borcheln, Germany	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: < 1 year. Weight: 271 – 481 g	
3.2.6	Number of animals per group	Untreated control	20 female
3.2.7	Control animals	Yes	

3.3 Administration/ Exposure

Intradermal

3.3.1	Induction schedule	day 1 Intradermal day 6 topical	
3.3.2	Way of Induction	Intradermal and topical	
3.3.3	Concentrations used for induction	5% NeemAzal T/S in sesame oil	
3.3.4	Concentration Freund's Complete Adjuvant (FCA)	1:1 FCA and water ad iniectionabilia	
3.3.5	Dermal induction	On day 6 the scapular area between the injection sites was clipped and rubbed with 0.5 mL of 10% sodium lauryl sulfate in vaseline– this concentration causes a mild inflammatory reaction. On day 7 the clipped area was treated with 1 mL of a undiluted NeemAzal T/S for 48 hours using a filter paper covered with impermeable plastic tape and fixed with an elastic adhesive bandage. The control animals were treated as described for the experimental animals except that, instead of the test substance, vehicle alone was administered.	
3.3.6	Challenge schedule	14 days after the last induction exposure (day 21)	
3.3.7	Concentrations used for challenge	25% NeemAzal T/S in sesame oil	
3.3.8	Rechallenge	No	
3.3.9	Scoring schedule	24h and 48h after challenge	
3.3.10	Removal of the test substance	After 24 h exposure the skin cleaned of residual test substance and vehicle using water	
3.3.11	Positive control substance	Ethyl p-aminobenzoate, test performed in parallel	
3.4	Examinations	Twice a day clinical signs were recorded and animals checked for mortality. Body weights were recorded on the first and last day of the study.	
3.4.1	Pilot study	Yes, a pretest was performed to identify concentrations for intradermal injections and epidermal applications	

Section B6.3**Skin sensitisation****Annex Point IIB-VI6.3**

Magnusson Kligman Test

3.5 Further remarks

None

4.2 Results of pilot studies

Upon epidermal application of NeemAzal in sesame oil(0.5 ml/site) severe erythema was noted at 100%, moderate erythema at 50 and no erythema at 25%. No oedema were observed.

There were no skin reactions observed upon intra dermal injection of 1%, 3% or 5% NeemAzal in sesame oil (0.1 mL/site).

4.3 Results of test

4.3.1 24h after challenge

No cutaneous reactions were observed.

4.3.2 48h after challenge

No cutaneous reactions were observed.

4.3.3 Other findings

A test with ethyl p-aminobenzoate as positive reference substance (performed in parallel during September 1998) resulted in allergic reactions and has shown the sensitivity of the guinea pig strain used.

4.4 Overall result

Under the conditions of this study, NeemAzal T/S did not exhibit a potential to produce dermal sensitisation in guinea pigs.

4 RESULTS AND DISCUSSION

Section B6.3**Skin sensitisation****Annex Point IIB-VI6.3**

Magnusson Kligman Test

5 APPLICANT'S SUMMARY AND CONCLUSION**5.2 Materials and methods**

Skin sensitisation potential of NeemAzal T/S was tested in a GLP study according to OECD guideline 406.

Experimental group (20 female animals): For induction, on day 1, the scapular region was clipped and the following three pairs of intradermal injections (0.1 mL/site) were made:

1:1 w/w mixture of Freund's Complete Adjuvant with water for injection

NeemAzal-T/S 5 % in sesame oil

NeemAzal-T/S 5 % in a 1:1 (v/v) mixture Freund's Complete Adjuvant and sesame oil.

On day 6 the scapular area between the injection sites was clipped and rubbed with 0.5 mL of 10% sodium lauryl sulfate in vaseline– this concentration causes a mild inflammatory reaction.

On day 7 the clipped area was treated with 1 mL of a undiluted NeemAzal T/S for 48 hours using a filter paper covered with impermeable plastic tape and fixed with an elastic adhesive bandage.

The control animals were treated as described for the experimental animals except that, instead of the test substance, vehicle alone was administered.

For challenge on day 21 both flanks of all animals were clipped and treated by epidermal application of 25 % NeemAzal-T/S in sesame oil (1 mL on the left flank) or sesame oil (right flank), using patch test plasters. The patches were held in place with tape and subsequently elastic bandage. The dressing was removed after 24 hours exposure and the skin cleaned of residual test substance and vehicle using water. The treated sites were assessed for challenge reactions 24 and 48 hours after removal of the dressing

5.3 Results and discussion

No mortality occurred and no symptoms of systemic toxicity were observed. Body weights and body weight gain remained in the same range as controls.

No skin reactions were observed in animals of the treatment group upon challenge with NeemAzal-T/S.

An earlier test with ethyl p-aminobenzoate as positive reference substance (performed in parallel during September 1998) resulted in allergic reactions and has shown the sensitivity of the guinea pig strain used.

5.4 Conclusion

The test substance NeemAzalT/S exhibited no dermal sensitization potential under the test conditions used according to Magnusson and Kligman. On the basis of this study NeemAzalT/S does not require labelling as sensitising.

5.4.1 Reliability

1

5.4.2 Deficiencies

No

Section B6.3**Skin sensitisation****Annex Point II B-VI 6.3**

Magnusson Kligman Test

Evaluation by Competent Authorities**EVALUATION BY RAPPORTEUR MEMBER STATE**

Date	2007/09/07
Reference	The applicant's version is adopted with exception of the following: Section 1.1 Kramer, H.-J. (1998) Skin Sensitisation Study according to Magnusson & Kligmar NeemAzal T/S BioChem, Karlsruhe, Germany Unpublished Report No. 981042830 Dates of experimental work: 11.09. – 10.10.1998
Materials and Methods	The applicant's version is adopted with exception of the following: Section 3.1.2.1 Brown liquid Section 3.1.2.2 1% Azadirachtin A (no certificate of analysis) Section 3.1.2.3 Expiry July 2000 Section 3.1.3 <u>for induction</u> : 5 % (intradermal) and 100 % (topical) <u>for challenge</u> : 25 % in sesame oil Section 3.2.2 Dunkin Hartley Section 3.2.4 Male and female Section 3.2.5 Age: < 1 year. Weight: 271 – 465 g Section 3.2.6 10 test substance 10 reference substance 5+5 control Section 3.3.1 Day 0 intradermal day 7 topical Section 3.3.3 5% NeemAzal T/S in sesame oil (intradermal) 100% NeemAzal T/S (topical) Section 3.3.9 24h and 48h after removal of the bandage (day 23, 24) Section 3.4 Clinical signs were recorded and animals checked for mortality. Body weights were recorded on days -5, -1, 0, 6, 7, 21, 23, 24 of the study.

Section B6.3**Skin sensitisation****Annex Point IIB-VI6.3****Magnusson Kligman Test****Results and discussion**

The applicant's version is adopted with exception of the following:

Section numbering is incorrect.

Section 4.1 Results of pilot studies

Upon epidermal application of NeemAzal in sesame oil (0.5 ml/site) severe erythema was noted at 100%, moderate erythema at 50% and no erythema at 25%. No oedema were observed. The suitable concentrations for the main study were 100% for topical induction and 25% for challenge.

Upon intradermal injection of 1% NeemAzal in sesame oil (0.1ml/site) no skin reactions were noted. At 3% mild erythema and at 5% moderate erythema but no oedema were observed. A concentration of 5% was chosen for the main study.

Conclusion

The following formal deviations were found in the applicant's version: Section numbering is incorrect.

Section 5.1 Material and methods (Second paragraph)

Experimental group (5 male + 5 female animals): For induction, on day 0, the scapular region was clipped and the following three pairs of intradermal injections (0.1 mL/site) were made:

Section 5.3.1 Reliability

2

Section 5.3.2 Deficiencies

Yes

With respect of contents RMS conclusions are differing from applicant's conclusions:

In the pretest, topical application of 100% NeemAzal T/S induced severe erythema. This concentration was chosen for dermal induction. Despite this irritation reaction, a pretreatment with 10% SDS was performed before dermal induction in the main study, a procedure which is usually performed only when the test substance is not irritating. Based on the results of the pretest, the reason for this remains unclear. Furthermore, the impact on the animals of the SDS-pretreatment followed by the undiluted test substance which alone caused severe erythema must be assumed to be a severe one. However, an inconsistency is found in the study report (p. 18, Causing of a local irritation). Here the reason for pretreatment with SDS is indicated as "No dermal reactions occurred in both groups." It is unclear where this observation comes from.

Impact on the study: When pretreatment with SDS has been performed, although not necessary, it can be regarded as a worst case and the results of the study can be judged as valid.

The test substance NeemAzalT/S exhibited no dermal sensitisation potential under the test conditions used according to Magnusson and Kligman. The test was performed only with 10 test and 5 control animals. However, according to OECD 406 it is strongly recommended to perform the test with 20 test and 10 control animals if the test substance is not a sensitiser. Therefore, a clear classification as not sensitising to skin basing on this study is not appropriate. However, the applicant submitted a previous study with the biocidal product. This study, which was considered reliable by RMS, was also negative. However, it was a Buehler-Test, which is only accepted in justified cases for classification. Nevertheless, this study strongly supports the results of the Magnusson-Kligman-Test leading to the conclusion that NeemAzalT/S does not require labelling as sensitising to skin.

Due to the above mentioned inconsistency in the study report and there from arising uncertainty, the reliability of the study has been set to 2.

Reliability

2

*Annex 1: Evaluation by the Rapporteur Member State, CA-Tables**CA-Table 1 –Table B6_3-1. Detailed information including induction/challenge/scoring schedule for skin sensitisation test*

<i>Inductions</i>	<i>GPMT</i>		<i>Observations/Remarks information on irritation effects</i>
	<i>day of treatment</i>	<i>application</i>	
<i>Induction 1</i>	<i>0</i>	<i>intradermal</i>	<i>not reported</i>
<i>pretreatment for non-irritating substances</i>	<i>6</i>	<i>0.5 ml 10 % SDS in vaseline</i>	<i>not reported</i>
<i>Induction 2</i>	<i>7</i>	<i>topical</i>	<i>not reported</i>
<i>challenge</i>	<i>21</i>	<i>topical</i>	<i>not reported</i>
<i>scoring 1</i>	<i>23</i>		<i>no skin reactions</i>
<i>scoring 2</i>	<i>24</i>		<i>no skin reactions</i>

CA-Table 2 - Table B6_3-2. Result of skin sensitisation test

	<i>Number of animals with signs of allergic reactions / number of animals in group</i>		
	<i>Negative control</i>	<i>Test group</i>	<i>Positive control</i>
<i>scored after 24h</i>	<i>0/5</i>	<i>0/10</i>	<i>8/10</i>
<i>scored after 48h</i>	<i>0/5</i>	<i>0/10</i>	<i>6/10</i>